

In the Specification:

Please enter replacement paragraphs in the specification as follows, where strikethroughs represent deleted text and underlining represents added text:

Please delete the paragraph on page 8, lines 19-30, and replace it with the following paragraph:

Three major sites of gp120 have been identified that are involved in cross-neutralization of diverse viral strains (Wyatt R. et al, Nature 393: 705-711, 1998). The V3 domain was found to express linear and conformational epitopes that can be recognized by antibodies that neutralize HIV-1. Although the V3 domain is a variable region, it contains a central portion shared by many HIV-1 isolates, particularly those found in the United States and Europe. The central portion has been called the principle neutralization epitope and is formed from a linear epitope of the amino acid sequence GP~~GRA~~FP (**SEQ ID NO: 28**)(Broliden P.A. et al, Proc. Natl. Acad. Sci. USA 89: 461-465, 1992; Broliden P.A. et al, Immunol. 73: 371-376, 1991; Javaherian K. et al, Science 250: 1590-1593, 1990; Javaherian K. et al, Proc. Natl. Acad. Sci. USA 86: 6768-6772, 1989). Conformational epitopes of the V3 loop have also been identified that can be recognized by antibodies that are more broadly neutralizing.

Please delete the paragraph on page 11, lines 11-27, and replace it with the following paragraph:

Figure 2.

A. Sequence of two cDNAs encoding HIV gp120-V3 loop/CD154 long form extracellular domain fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV V3 loop from gp120 with a (ProAspPro) linker (SEQUENCE ID NO.: 17 [DNA] OR SEQUENCE ID NO.: 25 [FUSION PROTEIN]) or a (Gly₄Ser)₃ (**SEQ ID NO: 29**) linker (SEQ. ID NO.: 16 [DNA] OR SEQ. ID NO.:24 [FUSION PROTEIN]) fused to the CD154

extracellular domain encoded between amino acids 48 (Arg)-261 (Leu), with an additional (Glu) residue at the carboxyl end of the protein, not present in wild type CD154. The sequence of the fusion protein is indicated using the three-letter amino acid code convention, above each codon of the open reading frame. Relevant restriction sites are indicated on the drawing and the nucleotides encoding sites at domain fusion junctions are displayed in boldface type, while the first codon of each fused domain is indicated in underlined, italicized type. The protein domains are labeled above the relevant position in the sequence. The nucleotide number is indicated in the left margin with a designation for the PDP linker form or the G4S linker form.

Please delete the paragraph on page 11, line 28, to page 12, line 4, and replace it with the following paragraph:

B. Sequence of two cDNAs encoding HIV V3 loop-CD154 short form extracellular domain fusion proteins.

The two HIV V3 loop constructs with alternate linkers, either (ProAspPro) (SEQUENCE ID NO.: 19 [DNA] OR SEQUENCE ID NO.: 27 [FUSION PROTEIN]) or (Gly₄Ser)₃ (**SEQ ID NO: 29** **linker**) (SEQUENCE ID NO.: 18 [DNA] OR SEQUENCE ID NO.: 26 [FUSION PROTEIN]) were also fused to the short form of the CD154 extracellular domain encoded from amino acids 108 (Glu)-261 (Leu) plus an extra glutamic acid residue at the carboxy terminus, not encoded by wild type CD154. All sequences are labeled as described for Figure 2A.

Please delete the paragraph on page 12, lines 5-13, and replace it with the following paragraph:

Figure 3.

A. Sequence of two HIV gp120env-CD154 long form extracellular domain cDNA and the predicted fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV gp120 with a (ProAspPro) linker (SEQ. ID NO.: 13 [DNA] OR SEQ. ID NO.: 21 [FUSION

PROTEIN]) or a (Gly₄Ser)₃ (**SEQ ID NO: 29**) linker (SEQ. ID NO.: 12 [DNA] OR SEQ. ID NO.: 20 [FUSION PROTEIN]) fused to the CD154 extracellular domain (Long Form) encoded between amino acids 48 (Arg)-261(Leu) + (Glu). All sequences are labeled as described for Figure 2A.

Please delete the paragraph on page 12, lines 14-21, and replace it with the following paragraph:

B. Sequence of two HIV gp120env-CD154 short form extracellular domain cDNAs and the predicted fusion proteins.

The sequence of a cDNA construct and corresponding fusion protein encoding the HIV gp120 with a (ProAspPro) linker (SEQ. ID NO.: 15 [DNA] or SEQ. ID NO.: 23 [fusion protein]) or a (Gly₄Ser)₃ (**SEQ ID NO: 29**) linker (SEQ. ID NO.: 14 [DNA] or SEQ. ID NO.: 22 [fusion protein]) fused to the short form of the CD154 extracellular domain encoded between amino acids 108 (Glu)-261 (Leu) + (Glu).. All sequences are labeled as described for Figure 2A.

Please delete the paragraph on page 16, lines 12-23, and replace it with the following paragraph:

The extracellular domain of human CD154 was PCR amplified using cDNA generated with random primers and RNA from human T lymphocytes activated with PHA (phytohemagglutinin). Two different fusion junctions were designed which resulted in a short or truncated form (form S4) including amino acids 108 (Glu)-261 (Leu) + (Glu),, and a long or complete form (form L2) including amino acids 48 (Arg) -261 (Leu) + (Glu), of the extracellular domain of CD154. The sense primer which fuses the extracellular domain to the targeted antigen includes a BamHI site for cloning that introduces the peptide sequence PDP or (ProAspPro) at the fusion junction and can also encode a linker peptide such as (Gly₄Ser)₃ (**SEQ ID NO: 29**) to separate the antigen from the extracellular domain. The oligonucleotide primers used in amplifying the short form (S4) of the CD154 extracellular domain encoding amino acids 108 (Glu)-261 (Leu) + (Glu) are as follows:

Please delete the paragraph on page 17, line 3, to page 18, line 3 and replace it with the following paragraph:

A variety of different antigens can be encoded on cDNA cassettes to be inserted between the leader peptide cassette and the CD40 targeted domain (such as a truncated or complete CD154 extracellular domain or a CD40 specific scFv). In a preferred embodiment of the invention, the cDNA antigen encoded by the vaccine is the HIV-1 gp 120 or a fragment of this antigen, such as the V3 loop. The primer sets used to amplify the complete gp120 domain include the sense primer SEQUENCE ID NO: 6 or GP120Bgl2f 5'-gga tat tga tga gat cta gtg cta cag-3' and one of two antisense primers encoding different linkers. Either the antisense primer encoding the ProAspPro linker, identified as SEQUENCE ID NO: 7 or GP120PDPr 5'-gaa cac agc tcc tat tgg atc cgg tct ttt ttc tct ttg cac-3' or the antisense primer encoding the (Gly₄Ser)₃ (**SEQ ID NO: 29**) linker, identified as SEQUENCE ID NO: 8 or GP120G4Sr 5'-cct gca tgg atc cga tcc gcc acc tcc aga acc tcc acc tcc tga acc gcc tcc ccc tct ttt ttc tct ttg cac tgt tct tct ctt tgc-3' were used to amplify the gp120 domain with the desired linker attached. PV75Kgp160(89.6) DNA was used as template in PCR reactions. Alternatively, other isolates or sequence variants of gp120 or gp160 are available and can be substituted to create novel fusion cassettes. PCR amplification reactions were performed using cloned plasmid DNA as template (approximately 45 ng), 3 mM MgCl₂, 0.3 mM dNTPs, 1/10 volume 10X reaction buffer supplied by the manufacturer, 10 pmol sense primer, 10 pmol antisense primer, and 2.5 units TAQ polymerase (Takara Pharmaceuticals) in a total reaction volume of 50 μ l. The amplification profile included an initial 4 minute 94°C denaturation, followed by a 30 cycle program of 50°C annealing for 30 seconds, 72°C extension for 30 seconds, and 94°C denaturation for 30 seconds. PCR fragments were purified by ethanol precipitation, resuspended in 30 μ l ddH₂O and 10 μ l was digested with BglII (Roche) restriction endonuclease in a 20 μ l reaction volume at 37°C for 3 hours. Fragments were gel purified, purified using QIAEX kits according to the manufacturer's instructions (QIAGEN, San Diego, CA), and ligated along with the annealed leader peptide oligonucleotides to HindIII-BamHI digested expression vector already containing the CD154 extracellular domain as a BamHI-XbaI fragment. Recombinant clones were screened for the correct orientation and presence of inserts, and the resulting positive clones were verified by

DNA sequencing using an ABI 310 sequence analyzer and the ABI Prism Dye Terminator Reaction Chemistry. The final fusion cassette encodes the synthetic leader peptide fused to the HIV gp120 domain with either a (ProAspPro) linker or a (Gly₄Ser)₃ (SEQ ID NO: 29) linker, and then to the CD154 extracellular domain long (Figure 3A) or short (Figure 3B) form to create the embodiments of example 1.

Please delete the paragraph on page 18, lines 5-11 and replace it with the following paragraph:

In an alternative preferred embodiment, the V1 and V2 domains of gp120 are removed and only the V3 loop domain from HIV gp 120 is encoded on a BglII-BamHI fragment and fused to the signal peptide and the CD154 extracellular domain to create the vaccine, as illustrated in Figure 2A and B. This antigen domain is separated from the CD154 short (Figure 2B) or long extracellular domain (Figure 2A) by a peptide linker encoding the amino acids (ProAspPro), or a longer peptide linker encoding the amino acids (Gly₄Ser)₃ (SEQ ID NO: 29).

Please delete the paragraph on page 18, lines 19-21 and replace it with the following paragraph:

The antisense primer encoding a (Gly₄Ser)₃ (SEQ ID NO: 29) linker is SEQUENCE ID NO: 11 or V3G4Sr

5'-ggg gca tgg atc cga acc tcc acc gcc aga tcc acc gcc tcc tga ggc acc gcc acc act aat gtt aca atg tgc ttg ttg tct tat atc tcc-3'.

Please delete the paragraph on page 18, lines 22-26 and replace it with the following paragraph:

Amplification, digestion, purification, and ligation conditions were identical to those described above for the full-length gp120 domain. The final fusion cassettes encode the HIV gp120-V3 loop with either a (ProAspPro) linker or a (Gly₄Ser)₃ (SEQ ID NO: 29) linker

fused to either the CD154 extracellular domain as diagrammed in Figure 2A for the long form, and Figure 2B for the short form of the CD40 binding domain.